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What are the progesterone-induced changes of the outcome and the serum markers of injury, oxidant activity and inflammation in diffuse axonal injury patients?

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ABSTRACT

To permit appropriate targeted therapy, the present clinical study was aimed to investigate the effects of progesterone on the outcome and the serum markers of injury, oxidant activity and inflammation in diffuse axonal injury (DAI). Forty-eight male DAI patients were divided into two groups (control and progesterone). Progesterone group received progesterone in dose of 1 mg/kg per 12 h for five days. The outcome was investigated using Extended Glasgow Outcome Scale (GOS-E) and functional independence measure (FIM). The markers of inflammation [interleukin-1β (IL-1β), IL-6, transforming growth factor-β1 (TGF-β1)], injury (brain protein of S-100B), and oxidant activity [malondialdehyde (MDA)] were evaluated in the serum of the patients. Higher GOS-E and FIM scores were observed in progesterone group at the six-month follow-up ($P < 0.05$ and $P < 0.01$, respectively). Meanwhile, a reduction in the serum levels of IL-1β, MDA and S-100B was noticed in progesterone group 24 h after injury ($P < 0.05$, $P < 0.001$ and $P < 0.05$, respectively), and there was an increase in serum levels of IL-6 and TGF-β1 ($P < 0.01$ and $P < 0.05$, respectively). Also, lower levels of MDA and S-100B, and higher levels of TGF-β1 were observed in progesterone group six days after injury ($P < 0.05$). According to these findings, progesterone may improve the outcome in DAI patients probably through modulation in the levels of cytokines, and reduction in the injury and oxidant activity.

1. Introduction

Traumatic brain injury (TBI) is a leading cause of death and severe disability around the world and results in large direct and indirect costs to society. Diffuse axonal injury (DAI) is one of the most common types of TBI, and accounts for about half of severe TBIs [1]. Although the clinical management of TBI has been greatly improved for the development of standards of care, no medical treatment has been proven to be effective in reducing death or disability following TBI [2].

TBI results in both primary brain injury immediately caused by an initial impact and secondary brain injury caused by cellular and molecular responses to a primary injury. These responses comprise releasing free radicals, neuroinflammation and apoptosis leading to brain edema and delayed neuronal death which are considered to worsen a primary brain injury and to influence the neurologic outcome of patients [3,4]. Therefore, major opportunity for interventions to limit neurological defect is reversing or preventing a secondary brain injury [5].

As secondary brain injury mechanisms are complex and various, simultaneous targeting of several injury factors using multipotential drugs may improve the outcome of TBI patients [6]. After three decades of extensive research on progesterone in TBI, it is known that this neurosteroid affects multiple mechanisms involved in neuroprotection and repair after various types of brain injury [7,8]. Although the clinical benefit of progesterone has been suggested in three phase II randomized controlled clinical trials for TBI patients [9–11], two phase III clinical trials have not displayed the efficacy of progesterone in TBI [12,13]. The adverse events attributable to progesterone drug have not been reported in TBI patients [10,11].

It seems that TBI studies should be focused on the molecular mechanisms of injury, rather than merely clinical observations. In recent years, TBI studies have been concentrated on whether brain-derived substances detectable in biological fluids could be useful as efficacy

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markers of therapeutic interventions [14]. A blood brain barrier (BBB) disruption causes either entering of peripheral proteins into cerebrospinal fluid (CSF) or leakage of CSF proteins that both can provide biomarkers of TBI [15]. Accordingly, S-100B is best known as a CSF/serum marker of injury in TBI [16,17], and is a calcium-binding protein physiologically produced and released by astrocytes in central nervous system (CNS) [18]. The concentration of this protein increases in CSF and serum following cerebral injuries [19], and can be useful as a serum biomarker of injury in DAI patients [20,21]. Malondialdehyde (MDA) is a noxious product of lipid peroxidation due to acting reactive oxygen species (ROS) which increase BBB permeability in TBI [22], moreover studies have reported a considerable increase in the production of ROS in TBI [23,24]. Because of short half-life of ROS, products of acting ROS including MDA are used for the estimation of ROS [25]. Damage to brain membrane lipids is an early event in brain injury [26]. An increase in brain levels of cytokines in patients with brain injury also causes neuroinflammation and damage of BBB leading to the releasing of cytokines into systemic blood circulation [3,27].

Progesterone reduces lipid peroxidation leading to the maintenance of membrane integrity and stabilization of BBB in experimental brain injury that improves the outcome [28–31]. Moreover, progesterone suppresses inflammation in preclinical models of TBI by modulating cytokine release and by inhibiting immune cell activation and migration [32–34]. Also, it has been previously indicated that progesterone reduces brain edema and BBB permeability following experimental TBI [35], and these effects were associated with reducing interleukin-1β (IL-1β), TNFα, IL-6 and increasing transforming growth factor-β1 (TGF-β1) in the brain [36,37].

Since the results of the performed clinical trials of progesterone in TBI are paradoxical, the effect of progesterone on the neurological outcome of DAI patients remains unknown [9–13]. Also, TBI biomarkers can indicate appropriate therapeutic strategies to minimize secondary brain injury and improve the development of individualized treatment, thereby reducing poor outcome [38]. Thus, considering the above, we designed a clinical trial to determine the effects of the early administration of progesterone on the outcome, and injury, oxidant activity and inflammation markers in moderate and severe DAI patients. To this end, firstly, the outcome was assessed using Extended-Glasgow Outcome Scale (GOS-E) and functional independence measure (FIM) at a three- and six-month follow-up. Secondly, injury (S-100B), oxidant activity (MDA) and inflammation (IL-1β, IL-6 and TGF-β1) markers were evaluated using serum collection at the time of admission, and 24 h and six days after injury.

2. Materials and methods

2.1. Patients and study design

The study conducting and reporting were according to Good Clinical Practice and CONSORT Guidelines [39] (Fig. 1). The study protocol was approved by ethics committee of Kerman University of Medical Sciences (K/92/579) and registered in Iranian Registry of Clinical Trials (www.irct.ir, CT2014042017356N1). This prospective, single-blind study was performed in the trauma main center of Kerman province, called Shahid Bahonar Hospital from May 2013 to July 2015.

The male patients with a non-penetrating TBI were selected on the basis of eligibility and exclusion criteria by a physician that was not informed to study design. The eligibility criteria were Glasgow Coma Scale (GCS) score of 12 or less, DAI using computed tomography (CT) scan, admission within four hours after injury, and 18 to 60 years of age. The exclusion criteria were a life expectancy of less than 24 h, a prolonged hypoxemia (partial pressure of arterial oxygen, <60 mmHg), hypotension (systolic blood pressure, <90 mmHg), selection for surgery, craniotomy, presence of other diseases and spinal cord injury at the time of randomization, and other traumas during DAI. An informed consent was taken from the patients' relatives.

In the current study, forty-eight male patients who were selected on the basis of eligibility and exclusion criteria were randomly placed in case (received progesterone) or control (DAI) groups. The sample size was estimated by PASS and NCSS software using values from relevant studies [11]. Randomization was performed using random digit

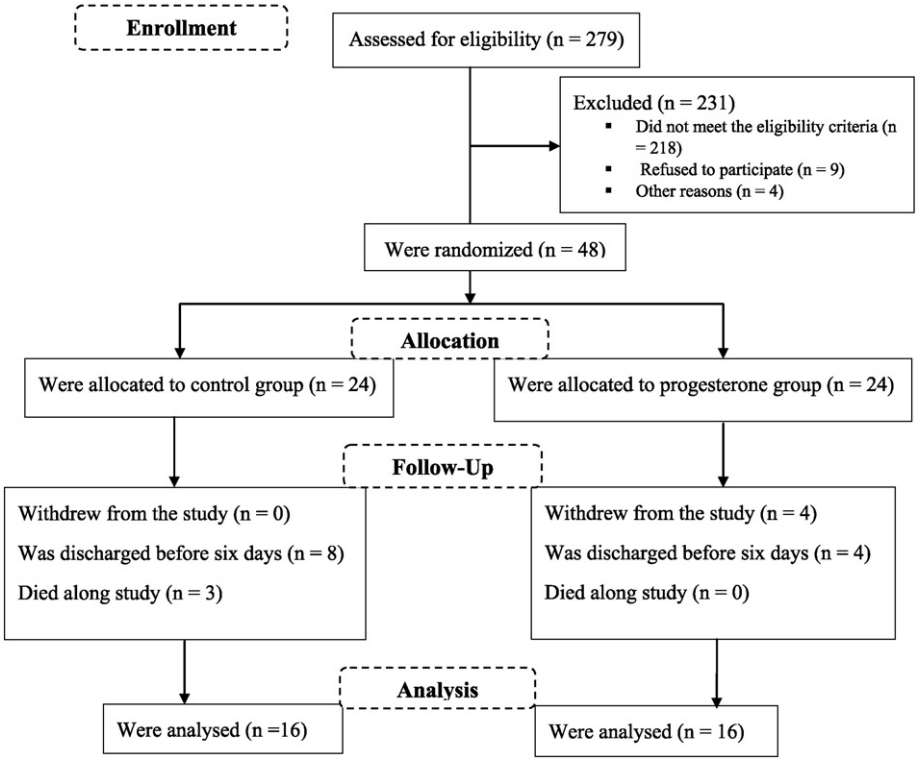

Fig. 1. Diagram of the phases of a parallel randomized trial for two groups of DAI patients (study enrollment, randomization, follow-up, and data analysis). n: number of patients.